

A/RE

Practitioner's Docket No. 05126.00002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Date: November 9, 2000

Assistant Commissioner for Patents
Washington, D.C. 20231

REISSUE APPLICATION TRANSMITTAL

Transmitted herewith is the application for reissue of U.S. Utility Patent No. 5,834,590 issued on November 10, 1998.

Inventors: Aaron I. Vinik; Gary L. Pittenger; Ronit Rafaeloff; Lawrence Rosenberg; and William P. Duguid

Title: INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS

Enclosed are the following:

1. Specification, claim(s) and drawing(s) (37 C.F.R. Section 1.173)

CERTIFICATION UNDER 37 C.F.R. SECTION 1.10*

(Express Mail label number is mandatory.)

(Express Mail certification is optional.)

I hereby certify that this Reissue Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date _____, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number _____, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. Section 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

***WARNING:** Each paper or fee filed by "Express Mail" must have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. Section 1.10(b).
"Since the filing of correspondence under [Section] 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

(Reissue Application Transmittal--page 1 of 6)

- Q**uestions are asked about the following:

3. Preliminary Amendment Attached

7 pages of declaration and power of attorney (partially executed)

5. Information Disclosure Statement Attached

Copies of the IDS citation(s) is/are attached.

6. Basic Filing Fee Calculation (37 C.F.R. Section 1.16(h), (i) and (j))

CLAIMS AS FILED			
Number Filed	Number Extra	Rate	Basic Fee (37 C.F.R. 1.16(h)) \$710.00
28	4	X \$18.00	\$72.00
Total Claims (37 C.F.R. 1.16(j))			
7	0	X \$80.00	\$0.00
Independent Claims (37 C.F.R. 1.16(i))			
Filing Fee Calculation			\$782.00

7. Small Entity Status

A statement that this filing is by a small entity is attached

Filing Fee Calculation (50% of above) \$391.00

8. Total Fees Due


Filing Fee	\$355.00
Claims in excess of original	36.00
Independent claims in excess of original	0.00
Total Fees Due	\$391.00

9. Method Of Payment of Fees

Payment deferred.

[illegible]

Date: Monday November 13, 2000


Signature of Practitioner

(Reissue Application Transmittal--page 4 of 6)

This is part of the application for a reissue patent filed herewith based on the original patent identified as follows:

Name of Patentee: Eastern Virginia Medical School of the Medical College of Hampton Roads
Patent Number: 5,834,590
Date Patent Issued: 11/10/1998
Title of Invention: INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS

I am an assignee owning an undivided interest to the above original patent.

I assent to the accompanying application for reissue.

Attached is a "Statement under 37 C.F.R. section 3.73(b) -- Establishing Right of Assignee to Take Action."

Eastern Virginia Medical School of the Medical College of Hampton Roads

[Signature]

Date: November 6, 2000

Signature of person signing for assignee

David E. Thiel
Vice President for Administration
& Finance



REISSUE APPLICATION BY ASSIGNEE, OFFER TO SURRENDER
(37 C.F.R. section 1.178)

To the Assistant Commissioner for Patents:

The undersigned makes this statement as part of the accompanying reissue application for the reissue of letters patent number 5,834,590, for an improvement in *INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS*, granted on November 10, 1998, to Aaron I. Vinik, Gary L. Pittenger, Ronit Rafaeloff, Lawrence Rosenberg, and William P. Duguid and declares that it is now owner by assignment of the entire interest in said original patent and hereby offers to surrender said letters patent.

STATEMENT BY ASSIGNEE

Attached is a "STATEMENT UNDER 37 C.F.R. section 3.73(b)," establishing the right of the assignee to take action in this reissue.

CERTIFICATION UNDER 37 C.F.R. section 1.10*

(Express Mail label number is mandatory.)

(Express Mail certification is optional.)

I hereby certify that this Reissue Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date _____, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number _____, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. section 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

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"Since the filing of correspondence under section 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

006111 5850250

of Hampton Roads

David Holt

Signature

[illegible]

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)	
Aaron I. Vinik et al.)	Group Art Unit:
Reissue of U.S. Patent No. 5,834,590)	Examiner:
Issued: November 10, 1998)	Atty. Dkt. No. 05126.00002

For: INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS

PRELIMINARY AMENDMENT

The Honorable Assistant Commissioner
For Patents
Washington, D.C. 20231

Sir:

Claims 1-24 were issued. Claims 3, 4, 14, and 15 are amended here. Claims 25-28 are added. Please enter the following amendments to the claims.

IN THE CLAIMS

3. (Amended) A preparation of a polypeptide which comprises a sequence of at least 15 consecutive amino acids of a naturally occurring mammalian islet neogenesis associated protein (INGAP protein), wherein said polypeptide has immunogenic activity and wherein said polypeptide is a portion of INGAP protein.

4. (Amended) [The preparation of claim 3 wherein said] A polypeptide which is a fusion of [said] (1) a sequence of at least 15 consecutive amino acids of a naturally occurring mammalian islet neogenesis associated protein (INGAP protein), wherein said polypeptide has immunogenic activity to (2) a second polypeptide derived from a second protein.

14. (Amended) A pharmaceutical composition comprising:

a preparation of a polypeptide which comprises a sequence of at least 15 consecutive amino acids of a naturally occurring mammalian islet neogenesis associated protein (INGAP protein) and a pharmaceutically acceptable diluent or carrier, wherein said polypeptide is capable of stimulating β cell regeneration of pancreatic ductal cells, and wherein said polypeptide is a portion of said INGAP protein.

15. (Amended) [The] A pharmaceutical composition [of claim 14] comprising:

a preparation of a polypeptide which is a fusion of (1) a [said] sequence of at least 15 consecutive amino acids of a naturally occurring mammalian islet neogenesis associated protein (INGAP protein) to (2) a second polypeptide derived from a second protein; and a pharmaceutically acceptable diluent or carrier, wherein said polypeptide is capable of stimulating β cell regeneration of pancreatic ductal cells.

Please add new claims 25-28.

25. The preparation of claim 1 wherein the INGAP protein is expressed in a host cell which comprises a vector which encodes the INGAP protein.

26. The preparation of claim 3 wherein said polypeptide is expressed in a host cell which comprises a vector which encodes the polypeptide.

27. The pharmaceutical composition of claim 12 wherein said INGAP protein is substantially free of other mammalian proteins.

28. The pharmaceutical composition of claim 14 wherein said polypeptide is substantially free of other mammalian protein.

REMARKS

Claims 3 and 14 have been amended to recite that the polypeptide is a portion of INGAP protein. This is supported *inter alia* at column 7, lines 32-47, and 47-67 where fragments of INGAP are disclosed.

Claims 4 and 15 are amended to make them independent. They incorporate explicitly the limitations of original claims 3 and 14, respectively.

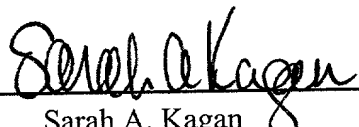
New claims 25-26 are dependent and specify that the protein is expressed in a host cell from a vector encoding INGAP. This is supported at column 7, lines 23-31 as well as at column 5, lines 63-66.

New claims 27 and 28 are dependent from claims 12 and 14 and recite a purity level which is substantially free of other mammalian proteins. This is supported *inter alia* at column 4, lines 15-20.

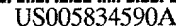
It is respectfully submitted that no new matter has been added.

Respectfully submitted,

Date: November 2, 2000

By: 
Sarah A. Kagan
Registration No. 32,141

Banner & Witcoff, Ltd.
1001 G Street, N.W., Eleventh Floor
Washington, D.C. 20001-4597
(202) 508-9100
SAK/ama



[11] Patent Number: 5,834,590

[45] **Date of Patent:** Nov. 10, 1998

- Rosenberg et al., "Reversal of Diabetes by the Induction of Islet Cell Neogenesis", *Transplantation Proceedings* 24(3):1027-1028 (1992).

Rosenberg L. et al. (1992) Pancreatic Islet Cell Regeneration and Growth. ed. Al Vinik, Plenum Press, New York, pp. 95-104, 1992.

Pittenger GL. et al. (1992) The Partial Isolation and Characterization of Iltropin, a Novel Islet-Specific Growth Factor (abstract) *Adv Exp Med Biol* 321, pp. 123-132, 1992.

Rouquier et al., "Rat Pancreatic Stone Protein Messenger RNA" *J. Biol. Chem.*, 266(2):786-791 (1991).

Lasserre et al., "A Novel Gene (HIP) Activated in Human Primary Liver Cancer", *Cancer Research* 52:5089-5095 (1992).

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Orelle et al., "Human Pancreatitis-associated Protein" *J. Clin. Invest.* 90:2284-2291 (1992).

U.S. PATENT DOCUMENTS

4,965,188 10/1990 Mullis et al. .

OTHER PUBLICATIONS

Stein et al., "Antisense Oligonucleotides as Therapeutic Agent—Is the Bullet Really Magical?", *Science* 261: 1004–1012 (1993).

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Miller et al., "Human Gene Therapy Comes of Age", *Nature* 357:455-460 (1992).

Watanabe et al., "Pancreatic Beta-Cell Replication and Amelioration of Surgical Diabetes by Reg Protein", *Proc. Natl. Acad. Sci. USA* 91:3589-3592 (1994).

Liang et al., "Distribution and Cloning of Eukaryotic mRNAs by Means of Differential Display: Refinements and Optimization", *Nucleic Acids Research* 21(14):3269-3275 (1993).

Primary Examiner—Robert A. Wax
Assistant Examiner—Enrique D. Longton
Attorney, Agent, or Firm—Banner & Witcoff, Ltd.

[57] **ABSTRACT**

Cellophane wrapping (CW) of hamster pancreas induces proliferation of duct epithelial cells followed by endocrine cell differentiation and islet neogenesis. Using the mRNA differential display technique a cDNA clone expressed in cellophane wrapped but not in control pancreata was identified. Using this cDNA as a probe, a cDNA library was screened and a gene not previously described was identified and named INGAP.

24 Claims, 4 Drawing Sheets

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Applicant or Patentee: **Aaron I. Vinik, Gary L. Pittenger, Ronit Rafaeloff, Lawrence Rosenberg and**

Attorney's #: **05126.00002**

William P. Duguid

Reissue of U.S. Patent No. 5,834,590

Filed or Issued: **Herewith**

For: **INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS**

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 C.F.R. §§1.9(f) AND 1.27(d)) — NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Eastern Virginia Medical School of the Medical College of Hampton Roads

ADDRESS OF ORGANIZATION: P.O. Box 1980, Norfolk, VA 23501-1980

TYPE OF ORGANIZATION

- ☒ UNIVERSITY OF OTHER INSTITUTION OF HIGHER EDUCATION
- ☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. §§501(a) and 501(c)(3))
- ☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA (NAME OF STATE) _____ (CITATION OF STATUTE _____)
- ☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. §§501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
- ☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA (NAME OF STATE) _____ (CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. §1.9(e) for purposes of paying reduced fees under §§41(a) and (b) of Title 35, United States Code, with regard to the invention entitled **INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS**

by inventor(s) Aaron I. Vinik, Gary L. Pittenger, Ronit Rafaeloff, Lawrence Rosenberg and William P. Duguid

described in ☐ the specification filed herewith.

☒ Reissue Application of 5,834,590 filed herewith

☐ patent no. _____ issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. §1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. §1.9(d) or a nonprofit organization under 37 C.F.R. §1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. §1.27)

FULL NAME McGill University

☐ Individual

ADDRESS 28 Place Richelieu

☐ Small Business Concern

Montreal, Quebec H3G 1E8, Canada

☒ Nonprofit Organization

FULL NAME GMP Companies, Inc.

☐ Individual

ADDRESS One East Broward Blvd, Suite 1701

☒ Small Business Concern

Fort Lauderdale, FL 33301

☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR §1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States Code; and further that false statements made willfully may jeopardize the validity of the application, of any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING David E. Thiel

TITLE IN ORGANIZATION Eastern Virginia Medical School Vice President for Administration and Finance

ADDRESS OF PERSON SIGNING P.O. Box 1980, Norfolk, Virginia 23501

SIGNATURE [Signature] DATE November 6, 2000

LAW OFFICES
BANNER & WITCOFF, LTD.
1001 G STREET, N.W.
WASHINGTON, D.C. 20001-4597
(202)508-9100

Revised May, 1992

Applicant or Patentee: Aaron I. Vinik, Gary L. Pittenger, Ronit Rafacloff, Attorney's #: 05126.00002
Lawrence Rosenberg and William P. Duguid
Reissue of Patent No.: 5,834,590 Filed or Issued: Herewith
For: INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 C.F.R. §§1.9(f) AND 1.27(c)) — SMALL BUSINESS CONCERN

I hereby declare that I am:

- ☐ the owner of the small business concern identified below;
☒ an official of the small business concern empowered to act on behalf of the concern identified below;

NAME OF CONCERN: GMP Companies, Inc.

ADDRESS OF CONCERN: One East Broward Blvd, Suite 1701
Fort Lauderdale, FL 33301

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR §§121.3-18, and reproduced in 37 CFR §1.9(d), for purposes of paying reduced fees under §§41(a) and (b) of Title 35, United States Code, in that the number of employees of the business concern (including those of its affiliates) does not exceed 500 persons. For purposes of this Statement: (1) the number of employees of the business concern is the average over the previous fiscal year of the business concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year; and (2) business concerns are affiliates of each other when either directly or indirectly one business concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled: INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS

by inventor(s) Aaron I. Vinik, Gary L. Pittenger, Ronit Rafacloff, Lawrence Rosenberg and William P. Duguid

described in ☐ the specification filed herewith.

☒ Reissue Patent No of 5,834,590 filed herewith

☐ patent no. _____ filed _____

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR §1.9(c) or by any concern which would not qualify as a small business concern under 37 C.F.R. §1.9(d) or a nonprofit organization under 37 C.F.R. §1.9(c). *NOTE: Separate verified statements are required from each named person, business concern or organization having rights to the invention averring to their status as small entities. (37 CFR §1.27)

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☒ Nonprofit Organization

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States Code; and further that false statements made willfully may jeopardize the validity of the application, of any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Jeffrey L. Raney

TITLE OF PERSON (other than owner) Secretary & General Counsel

ADDRESS OF PERSON SIGNING One East Broward Blvd., Suite 1701, Ft. Lauderdale, FL 33301

SIGNATURE 

DATE November 2, 2000

LAW OFFICES
BANNER & WITCOFF, LTD.
1001 G STREET, N.W.
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Revised May, 1992

FIG. 1A

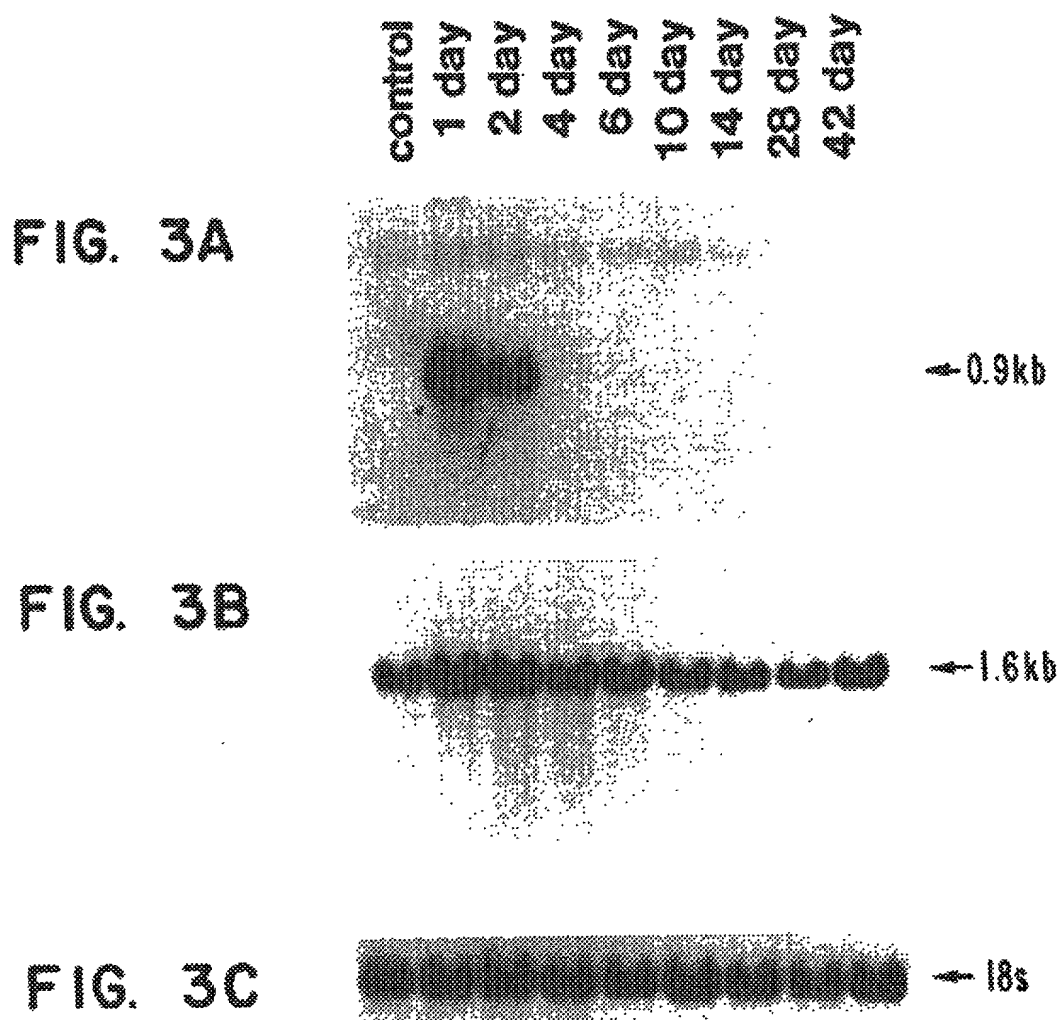
CTGCAAGACA GGTACCATG ATG CTT CCC ATG ACC CTC TGT AGG ATG TCT TGG	52
Met Leu Pro Met Thr Leu Cys Arg Met Ser Trp	10
ATG CTG CTT TCC TGC CTG ATG TTC CTT TCT TGG GTG GAA GGT GAA GAA	100
Met Leu Leu Ser Cys Leu Met Phe Leu Ser Trp Val Glu Gly Glu Glu	25
TCT CAA AAG AAA CTG CCT TCT TCA CGT ATA ACC TGT CCT CAA GGC TCT	148
Ser Gln Lys Lys Leu Pro Ser Ser Arg Ile Thr Cys Pro Gln Gly Ser	40
GTA GCC TAT GGG TCC TAT TGC TAT TCA CTG ATT TTG ATA CCA CAG ACC	196
Val Ala Tyr Gly Ser Tyr Cys Tyr Ser Leu Ile Leu Ile Pro Gln Thr	55
TGG TCT AAT GCA GAA CTA TCC TGC CAG ATG CAT TTC TCA GGA CAC CTG	244
Trp Ser Asn Ala Glu Leu Ser Cys Gln Met His Phe Ser Gly His Leu	75
GCA TTT CTT CTC AGT ACT GGT GAA ATT ACC TTC GTG TCC TCC CTT GTG	292
Ala Phe Leu Leu Ser Thr Thr Gly Glu Ile Thr Phe Val Ser Ser Leu Val	90
AAG AAC AGT TTG ACG GCC TAC CAG TAC ATC TGG ATT GGA CTC CAT GAT	340
Lys Asn Ser Leu Thr Ala Tyr Gln Tyr Ile Trp Ile Gly Leu His Asp	105

FIG. 1B

GGCTGTAACC TAAAGGCTCA GAGACAAAA ATAAAATGTC ATCAAC

[illegible]

	*	*	*
INGAP	YCAVLSQKSGFQKWRDFNCENELPYICKFKV	175	
PAP-I	FCGSLSRSSGFLRWRDTTCEVKLPYVCKFTG	176	
PAP-H/HIP	HCA SLRSTAF LRWKDYNCNVR LPYVCKFTD	176	
PAP-III	HCGTLTRASGFLRWRENNCISELPYVCKFKA	175	
PAP-II	NCGSLTATSEFLKWGDHCDVELPFVCKFKQ	175	
REG/LITH	YCVSVTSNSGYKKWRD NSCDAQLSFVCKFKA	165	
"DRICKAMER"	EC	G	WND C CE



BACKGROUND OF THE INVENTION

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6:

It is an object of the invention to provide antisense constructs for regulating the expression of INGAP.

It is still another object of the invention to provide kits for detecting mammalian INGAP proteins.

These and other objects of the invention are provided by one or more of the embodiments described below.

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6:

According to another embodiment of the invention a method of treating isolated islet cells of a mammal to avoid apoptosis of said cells is provided. The method comprises:

a mammalian INGAP protein in a pharmaceutically acceptable diluent or carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

If two oligonucleotides are hybridized to the genomic DNA or cDNA of the mammal then they can be used as primers for DNA synthesis, for example using the polymerase chain reaction or the ligase chain reaction. Construction of a full-length gene and confirmation of the identity of the isolated gene can be performed as described above.

INGAP protein may be isolated according to the invention by inducing mammalian pancreatic cells to express INGAP protein by means of cellophane-wrapping. This technique is described in detail in reference no. 1 which is expressly incorporated herein. Briefly, the pancreas is exposed and a strip of sterile cellophane tape is wrapped carefully around the head of the gland, so as not to crush the underlying tissue. Duct ligation is not involved. INGAP protein so produced may be purified from other mammalian proteins by means of immunoaffinity techniques, for example, or other techniques known in the art of protein purification. An antibody specific for a mammalian INGAP is produced using all, or fragments of, the amino acid sequence of an INGAP protein, such as shown in SEQ ID NO: 2, as immunogens. The immunogens can be used to identify and purify immunoreactive antibodies. Monoclonal or polyclonal antibodies can be made as is well known in the art. The antibodies can be conjugated to other moieties, such as detectable labels or solid support materials. Such antibodies can be used to purify proteins isolated from mammalian pancreatic cells or from recombinant cells. Hybridomas which secrete specific antibodies for an INGAP protein are also within the contemplation of the invention.

Host cells as described above can be used to produce a mammalian INGAP protein. The host cells comprise a DNA molecule encoding a mammalian INGAP protein. The DNA can be according to SEQ ID NO:1, or isolated from other mammals according to methods described above. Host cells can be cultured in a nutrient medium under conditions where INGAP protein is expressed. INGAP protein can be isolated from the host cells or the nutrient medium, if the INGAP protein is secreted from the host cells.

It has now been found that INGAP and fragments thereof are capable of inducing and stimulating islet cells to grow. Moreover, they are capable of inducing differentiation of pancreatic duct cells, and of allowing such cells to avoid the apoptotic pathway. Thus many therapeutic modalities are now possible using INGAP, fragments thereof, and nucleotide sequences encoding INGAP. Therapeutically effective amounts of INGAP are supplied to patient pancreata, to isolated islet cells, and to encapsulated pancreatic islet cells, such as in a polycarbon shell. Suitable amounts of INGAP for therapeutic purposes range from 1–150 $\mu\text{g/kg}$ of body weight or in vitro from 1–10,000 $\mu\text{g/ml}$. Optimization of such dosages can be ascertained by routine testing. Methods of administering INGAP to mammals can be any that are known in the art, including subcutaneous, via the portal vein, by local perfusion, etc.

Conditions which can be treated according to the invention by supplying INGAP include diabetes mellitus, both insulin dependent and non-insulin dependent, pancreatic insufficiency, pancreatic failure, etc. Inhibition of INGAP expression can be used to treat nesidioblastosis.

According to the present invention, it has now been found that a small portion of INGAP is sufficient to confer biological activity. A fragment of 20 amino acids of the sequence of SEQ ID NO: 2, from amino acid #103–#122 is sufficient to stimulate pancreatic ductal cells to grow and proliferate. The effect has been seen on a rat tumor duct cell line, a hamster duct cell line, a hamster insulinoma cell line, and a rat insulinoma cell line. The analogous portions of other mammalian INGAP proteins are quite likely to have the same activity. This portion of the protein is not similar to other members of the pancreatitis associated protein (PAP) family of proteins. It contains a glycosylation site and it is likely to be a primary antigenic site of the protein as well. This fragment has been used to immunize mice to generate monoclonal antibodies.

The physiological site of expression of INGAP has been determined. INGAP is expressed in acinar tissue, in the exocrine portion of the pancreas. It is not expressed in ductal or islet cells, i. e., the paracrine portion of the pancreas. Expression occurs within 24–48 hours of induction by means of cellophane wrapping.

Transgenic animals according to the present invention are mammals which carry an INGAP gene from a different mammal. The transgene can be expressed to a higher level than the endogenous INGAP genes by judicious choice of transcription regulatory regions. Methods for making transgenic animals are well-known in the art, and any such method can be used. Animals which have been genetically engineered to carry insertions, deletions, or other mutations which alter the structure of the INGAP protein or regulation of expression of INGAP are also contemplated by this invention. The techniques for effecting these mutations are known in the art.

Diagnostic assays are also contemplated within the scope of the present invention. Mutations in INGAP can be ascertained in samples such as blood, amniotic fluid, chorionic villus, blastocyst, and pancreatic cells. Such mutations identify individuals who are at risk for diabetes. Mutations can be identified by comparing the nucleotide sequence to a wild-type sequence of an INGAP gene. This can be accomplished by any technique known in the art, including comparing restriction fragment length polymorphisms, comparing polymerase chain reaction products, nuclease protection assays, etc. Alternatively, altered proteins can be identified, e.g., immunologically or biologically.

The present invention also contemplates the use of INGAP antisense constructs for treating nesidioblastosis, a condition characterized by overgrowth of β cells. The antisense construct is administered to a mammal having nesidioblastosis, thereby inhibiting the overgrowth of β cells. An antisense construct typically comprises a promoter, a terminator, and a nucleotide sequence consisting of a mammalian INGAP gene. The INGAP sequence is between the promoter and the terminator and is inverted with respect to the promoter as it is expressed naturally. Upon expression from the promoter, an mRNA complementary to native mammalian INGAP is produced.

Immunological methods for assaying INGAP in a sample from a mammal are useful, for example, to monitor the therapeutic administration of INGAP. Typically an antibody specific for INGAP will be contacted with the sample and the binding between the antibody and any INGAP in the sample will be detected. This can be by means of a competitive binding assay, in which the incubation mixture is spiked with a known amount of a standard INGAP preparation, which may conveniently be detectably labeled. Alternatively, a polypeptide fragment of INGAP may be used as a competitor. In one particular assay format, the antibodies are bound to a solid phase or support, such as a bead, polymer matrix, or a microtiter plate.

According to the present invention, pancreatic duct cells of a mammal with pancreatic endocrine failure can be removed from the body and treated in vitro. The duct cells typically comprise β cell progenitors. Thus treatment with a preparation of a mammalian INGAP protein will induce differentiation of the β cell progenitors. The duct cells are contacted with a preparation of a mammalian INGAP protein substantially free of other mammalian proteins. The treated cells can then be used as an autologous transplant into the mammal from whom they were derived. Such an autologous treatment minimizes adverse host versus graft reactions involved in transplants.

INGAP protein can also be used to identify those cells which bear receptors for INGAP. Such cells are likely to be the β cell progenitors, which are sensitive to the biological effects of INGAP. INGAP protein can be detectably labeled, such as with a radiolabel or a fluorescent label, and then contacted with a population of cells from the pancreatic duct. Cells which bind to the labeled protein will be identified as those which bear receptors for INGAP, and thus are β cell progenitors. Fragments of INGAP can also be used for this purpose, as can immobilized INGAP which can be used to separate cells from a mixed population of cells to a solid support. INGAP can be immobilized to solid phase or support by adsorption to a surface, by means of an antibody, or by conjugation. Any other means as is known in the art can also be used.

Kits are provided by the present invention for detecting a mammalian INGAP protein in a sample. This may be useful, inter alia, for monitoring metabolism of INGAP during therapy which involves administration of INGAP to a mammal. The kit will typically contain an antibody preparation which is specifically immunoreactive with a mammalian INGAP protein. The antibodies may be polyclonal or monoclonal. If polyclonal they may be affinity purified to render them monospecific. The kit will also typically contain a polypeptide which has at least 15 consecutive amino acids of a mammalian INGAP protein. The polypeptide is used to compete with the INGAP protein in a sample for binding to the antibody. Desirably the polypeptide will be detectably labeled. The polypeptide will contain the portion of INGAP to which the antibody binds. Thus if the antibody is monoclonal, the polypeptide will successfully compete with INGAP by virtue of it containing the epitope of the antibody. It may also be desirable that the antibodies be bound to a solid phase or support, such as polymeric beads, sticks, plates, etc.

Pharmaceutical compositions containing a mammalian INGAP protein may be used for treatment of pancreatic insufficiency. The composition may alternatively contain a polypeptide which contains a sequence of at least 15 consecutive amino acids of a mammalian INGAP protein. The polypeptide will contain a portion of INGAP which is biologically active in the absence of the other portions of the protein. The polypeptide may be part of a larger protein, such as a genetic fusion with a second protein or polypeptide. Alternatively, the polypeptide may be conjugated to a second protein, for example, by means of a cross-linking agent. Suitable portions of INGAP proteins may be determined by homology with amino acids #103 to #122 of SEQ ID NO:2, or by the ability of test polypeptides to stimulate pancreatic duct cells to grow and proliferate. As is known in the art, it is often the case that a relatively small number of amino acids can be removed from either end of a protein without destroying activity. Thus it is contemplated within the scope of the invention that up to about 10% of the protein can be deleted, and still provide essentially all functions of INGAP. Such proteins have at least about 130 amino acids, in the case of hamster INGAP.

The pharmaceutical composition will contain a pharmaceutically acceptable diluent or carrier. A liquid formulation is generally preferred. INGAP may be formulated at different concentrations or using different formulants. For

example, these formulants may include oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di-, or polysaccharides, or water soluble glucans. The saccharides or glucans can include fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. Sucrose is most preferred. Sugar alcohol is defined as a C_4 to C_8 hydrocarbon having an $-OH$ group and includes galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. Mannitol is most preferred. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. Preferably, the sugar or sugar alcohol concentration is between 1.0 w/v % and 7.0 w/v %, more preferable between 2.0 and 6.0 w/v %. Preferably amino acids include levorotary (L) forms of camitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. It is also preferred to use a buffer in the composition to minimize pH changes in the solution before lyophilization or after reconstitution, if these are used. Most any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are preferred. Preferably, the concentration is from 0.01 to 0.3 molar. Surfactants can also be added to the formulation.

Additionally, INGAP or polypeptide portions thereof can be chemically modified by covalent conjugation to a polymer to increase its circulating half-life, for example. Preferred polymers, and methods to attach them to peptides, are shown in U.S. Pat. Nos. 4,766,106, 4,179,337, 4,495,285, and 4,609,546. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O-CH_2-CH_2)_nO-R$ where R can be hydrogen, or a protective group such as an alkyl or alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1000 and 40,000, more preferably between 2000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor.

After the liquid pharmaceutical composition is prepared, it is preferably lyophilized to prevent degradation and to preserve sterility. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) which may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

The following examples are not intended to limit the scope of the invention, but merely to exemplify that which is taught above.

EXAMPLES

Example 1

This example describes the cloning and isolation of a cDNA encoding a novel, developmentally regulated, pancreatic protein.

We hypothesized that a unique locally produced factor(s) is responsible for islet cell regeneration. Using the recently developed mRNA differential display technique (5,6) to compare genes differentially expressed in cellophane wrapped (CW) versus control pancreata (CP) allowed us to identify a cDNA clone (RD19-2) which was uniquely expressed in cellophane wrapped pancreas.

A cDNA library was constructed from mRNA isolated from cellophane wrapped hamster pancreas using oligo d(T) primed synthesis, and ligation into pcDNA3 vector (Invitrogen). The number of primary recombinants in the library was 1.2×10^6 with an average size of 1.1 kb. The cDNA library was screened for clones of interest using high density colony plating techniques. Colonies were lifted onto nylon membranes (Schleicher & Schuell) and further digested with proteinase K (50(g/ml). Treated membranes were baked at 80° C. for 1 hour and hybridized at 50° C. for 16–18 hours with $1-5 \times 10^6$ cpm/ml of [32 P]-dCTP (Dupont-NewEngland Nuclear) radiolabeled RD19-2 probe. Colonies with a positive hybridization signal were isolated, compared for size with Northern mRNA transcript, and sequenced to confirm identity with the RD19-2 sequence.

Example 2

This example compares the sequence of INGAP to other proteins with which it shares homology.

The nucleotide sequence of the hamster INGAP clone with the longest cDNA insert was determined. As shown in FIGS. 1A and 1B the hamster cDNA comprises 747 nucleotides (nt), exclusive of the poly(A) tail and contains a major open reading frame encoding a 175 amino acid protein. The open reading frame is followed by a 3'-untranslated region of 206 nt. A typical polyadenylation signal is present 11 nt upstream of the poly(A) tail. The predicted INGAP protein shows structural homology to both the PAP/HIP family of genes which is associated with pancreatitis or liver adenocarcinoma (7–11) and the Reg/PSP/lithostatine family of genes (13,15) which has been shown to stimulate pancreatic beta-cell growth (14) and might play a role in pancreatic islet regeneration. Comparison of the nucleotide sequence and their deduced amino acids between hamster INGAP and rat PAP-I shows a high degree of homology in the coding region (60 and 58 % in nucleotide and amino acid sequences, respectively). The predicted amino acid sequence of the hamster INGAP reveals 45 % identity to PAP II and 50% to PAP III both of which have been associated with acute pancreatitis, and 54% to HIP which was found in a hepatocellular carcinoma. INGAP also shows 40% identity to the rat Reg/PSP/lithostatine protein (FIG. 2). Reg is thought to be identical to the pancreatic stone protein (PSP) (15,16) or pancreatic thread protein (PTP) (17). The N-terminus of the predicted sequence of INGAP protein is highly hydrophobic which makes it a good candidate for being the signal peptide which would allow the protein to be secreted. Similar to PAP/HIP but different from the Reg/PSP/lithostatine proteins a potential N-glycosylation site is situated at position

135 of the INGAP sequence. Unique to INGAP is another potential N-glycosylation site situated at position 115. INGAP also shows a high degree of homology (12/18) (FIG. 2) with a consensus motif in members of the calcium-dependent (C-type) animal lectin as determined by Drickamer including four perfectly conserved cysteines which form two disulfide bonds (12). Two extra cysteines found at the amino-terminus of INGAP (FIG. 2) are also present in Reg/PSP and PAP/HIP. However, it is not clear what the biological significance might be.

Example 3

This example demonstrates the temporal expression pattern of INGAP upon cellophane-wrapping.

In order to determine the temporal expression of the INGAP gene, total RNA extracted from CP and CW pancreas was probed with the hamster INGAP cDNA clone in Northern blot analysis. A strong single transcript of 900 bp was detected (FIGS. 3A, 3B and 3C) 1 and 2 days after cellophane wrapping which disappeared by 6 through 42 days and was absent from CP. INGAP mRNA is associated with CW induced pancreatic islet neogenesis, since it is present only after CW. It is not likely that the increased expression of INGAP is associated with acute pancreatitis as is the case with the PAP family of genes. During the acute phase of pancreatitis the concentrations of most mRNAs encoding pancreatic enzymes including amylase are decreased significantly (16,18). In contrast, in the CW model of islet neogenesis in which high expression of INGAP has been detected, amylase gene expression was simultaneously increased above normal (FIGS. 3A, 3B and 3C) rather than decreased, suggesting that INGAP expression is not associated with pancreatitis but rather with islet neogenesis. The cause of increased amylase gene expression 1 and 2 days after CW is as yet unclear, and more studies need to be done to elucidate this issue. It is unlikely though, that the increase is associated with exocrine cell regeneration which occurs at a later time after CW (19). Thus, INGAP protein plays a role in stimulation of islet neogenesis, in particular, in beta cell regeneration from ductal cells.

References

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 7

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 747 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Cricetulus

(i x) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 20..541

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTGCAAGACA	GGTACCATG	ATG	CTT	CCC	ATG	ACC	CTC	TGT	AGG	ATG	TCT	TGG	52			
		Met	Leu	Pro	Met	Thr	Leu	Cys	Arg	Met	Ser	Trp				
		1				5					10					
ATG	CTG	CTT	TCC	TGC	CTG	ATG	TTC	CTT	TCT	TGG	GTG	GAA	GGT	GAA	GAA	100
Met	Leu	Leu	Ser	Cys	Leu	Met	Phe	Leu	Ser	Trp	Val	Gln	Gly	Gln	Glu	
			15					20				25				
TCT	CAA	AAG	AAA	CTG	CCT	TCT	TCA	CGT	ATA	ACC	TGT	CCT	CAA	GGC	TCT	148
Ser	Gln	Lys	Lys	Leu	Pro	Ser	Ser	Arg	Ile	Thr	Cys	Pro	Gln	Gly	Ser	
		30					35				40					
GTA	GCC	TAT	GGG	TCC	TAT	TGC	TAT	TCA	CTG	ATT	TTG	ATA	CCA	CAG	ACC	196
Val	Ala	Tyr	Gly	Ser	Tyr	Cys	Tyr	Ser	Leu	Ile	Leu	Ile	Pro	Gln	Thr	
	45					50					55					
TGG	TCT	AAT	GCA	GAA	CTA	TCC	TGC	CAG	ATG	CAT	TTC	TCA	GGA	CAC	CTG	244
Trp	Ser	Asn	Ala	Glu	Leu	Ser	Cys	Gln	Met	His	Phe	Ser	Gly	His	Leu	
	60				65				70					75		
GCA	TTT	CTT	CTC	AGT	ACT	GGT	GAA	ATT	ACC	TTC	GTG	TCC	TCC	CTT	GTG	292
Ala	Phe	Leu	Leu	Ser	Thr	Gly	Glu	Ile	Thr	Phe	Val	Ser	Ser	Leu	Val	
			80					85						90		
AAG	AAC	AGT	TTG	ACG	GCC	TAC	CAG	TAC	ATC	TGG	ATT	GGA	CTC	CAT	GAT	340
Lys	Asn	Ser	Leu	Thr	Ala	Tyr	Gln	Tyr	Ile	Trp	Ile	Gly	Leu	His	Asp	
		95					100					105				
CCC	TCA	CAT	GGT	ACA	CTA	CCC	AAC	GGA	AGT	GGA	TGG	AAG	TGG	AGC	AGT	388
Pro	Ser	His	Gly	Thr	Leu	Pro	Asn	Gly	Ser	Gly	Trp	Lys	Trp	Ser	Ser	
		110					115					120				
TCC	AAT	GTG	CTG	ACC	TTC	TAT	AAC	TGG	GAG	AGG	AAC	CCC	TCT	ATT	GCT	436
Ser	Asn	Val	Leu	Thr	Phe	Tyr	Asn	Trp	Glu	Arg	Asn	Pro	Ser	Ile	Ala	
	125					130					135					
GCT	GAC	CGT	GGT	TAT	TGT	GCA	GTT	TTG	TCT	CAG	AAA	TCA	GGT	TTT	CAG	484

16

[illegible]

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 174 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met 1	Leu	Pro	Met	Thr 5	Leu	Cys	Arg	Met	Ser 10	Trp	Met	Leu	Leu	Ser 15	Cys
Leu	Met	Phe	Leu 20	Ser	Trp	Val	Glu	Gly 25	Glu	Glu	Ser	Gln	Lys 30	Lys	Leu
Pro	Ser	Ser 35	Arg	Ile	Thr	Cys	Pro 40	Gln	Gly	Ser	Val	Ala 45	Tyr	Gly	Ser
Tyr	Cys 50	Tyr	Ser	Leu	Ile	Leu 55	Ile	Pro	Gln	Thr	Trp 60	Ser	Asn	Ala	Gln
Leu 65	Ser	Cys	Gln	Met	His 70	Phe	Ser	Gly	His	Leu 75	Ala	Phe	Leu	Leu	Ser 80
Thr	Gly	Glu	Ile	Thr 85	Phe	Val	Ser	Ser	Leu 90	Val	Lys	Asn	Ser	Leu 95	Thr
Ala	Tyr	Gln	Tyr 100	Ile	Trp	Ile	Gly	Leu 105	His	Asp	Pro	Ser	His 110	Gly	Thr
Leu	Pro	Asn 115	Gly	Ser	Gly	Trp	Lys 120	Trp	Ser	Ser	Ser	Asn 125	Val	Leu	Thr
Phe	Tyr 130	Asn	Trp	Glu	Arg	Asn 135	Pro	Ser	Ile	Ala	Ala 140	Asp	Arg	Gly	Tyr
Cys 145	Ala	Val	Leu	Ser	Gln 150	Lys	Ser	Gly	Phe	Gln 155	Lys	Trp	Arg	Asp	Phe 160
Asn	Cys	Glu	Asn	Glu 165	Leu	Pro	Tyr	Ile	Cys 170	Lys	Phe	Lys	Val		

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i v) ANTI-SENSE: NO

(A) ORGANISM: *Rattus rattus*

Met Leu His Arg Leu Ala Phe Pro Val Met Ser Trp Met Leu Leu Ser
1 5 10 15

-continued

Cys	Leu	Met	Leu	Leu	Ser	Gln	Val	Gln	Gly	Glu	Asp	Ser	Pro	Lys	Lys
			20					25					30		
Ile	Pro	Ser	Ala	Arg	Ile	Ser	Cys	Pro	Lys	Gly	Ser	Gln	Ala	Tyr	Gly
		35					40					45			
Ser	Tyr	Cys	Tyr	Ala	Leu	Phe	Gln	Ile	Pro	Gln	Thr	Trp	Phe	Asp	Ala
	50					55					60				
Glu	Leu	Ala	Cys	Gln	Lys	Arg	Pro	Glu	Gly	His	Leu	Val	Ser	Val	Leu
65					70					75					80
Asn	Val	Ala	Glu	Ala	Ser	Phe	Leu	Ala	Ser	Met	Val	Lys	Asn	Thr	Gly
				85					90					95	
Asn	Ser	Tyr	Gln	Tyr	Ile	Trp	Ile	Gly	Leu	His	Asp	Pro	Thr	Leu	Gly
			100					105					110		
Gly	Glu	Pro	Asn	Gly	Gly	Gly	Trp	Glu	Trp	Ser	Asn	Asn	Asp	Ile	Met
		115					120					125			
Asn	Tyr	Val	Asn	Trp	Glu	Arg	Asn	Pro	Ser	Thr	Ala	Leu	Asp	Arg	Gly
	130					135					140				
Phe	Cys	Gly	Ser	Leu	Ser	Arg	Ser	Ser	Gly	Phe	Leu	Arg	Trp	Arg	Asp
145					150					155					160
Thr	Thr	Cys	Glu	Val	Lys	Leu	Pro	Tyr	Val	Cys	Lys	Phe	Thr	Gly	
				165					170					175	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 175 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Leu	Pro	Pro	Met	Ala	Leu	Pro	Ser	Val	Ser	Trp	Met	Leu	Leu	Ser
1				5					10					15	
Cys	Leu	Met	Leu	Leu	Ser	Gln	Val	Gln	Gly	Glu	Glu	Pro	Gln	Arg	Glu
		20						25					30		
Leu	Pro	Ser	Ala	Arg	Ile	Arg	Cys	Pro	Lys	Gly	Ser	Lys	Ala	Tyr	Gly
		35					40					45			
Ser	His	Cys	Tyr	Ala	Leu	Phe	Leu	Ser	Pro	Lys	Ser	Trp	Thr	Asp	Ala
	50					55					60				
Asp	Leu	Ala	Cys	Gln	Lys	Arg	Pro	Ser	Gly	Asn	Leu	Val	Ser	Val	Leu
65				70						75					80
Ser	Gly	Ala	Glu	Gly	Ser	Phe	Val	Ser	Ser	Leu	Val	Lys	Ser	Ile	Gly
			85					90						95	
Asn	Ser	Tyr	Ser	Tyr	Val	Trp	Ile	Gly	Leu	His	Asp	Pro	Thr	Gln	Gly
		100						105					110		
Thr	Glu	Pro	Asn	Gly	Glu	Gly	Trp	Glu	Trp	Ser	Ser	Ser	Asp	Val	Met
		115					120					125			
Asn	Tyr	Phe	Ala	Trp	Glu	Arg	Asn	Pro	Ser	Thr	Ile	Ser	Ser	Pro	Gly
	130					135					140				
His	Cys	Ala	Ser	Leu	Ser	Arg	Ser	Thr	Ala	Phe	Leu	Arg	Trp	Lys	Asp
145					150					155					160
Tyr	Asn	Cys	Asn	Val	Arg	Leu	Pro	Tyr	Val	Cys	Lys	Phe	Thr	Asp	
				165					170					175	

(2) INFORMATION FOR SEQ ID NO:5:

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:
(A) ORGANISM: *Rattus rattus*

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

(2) INFORMATION FOR SEQ ID NO:6:

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:
(A) ORGANISM: *Rattus rattus*

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met 1	Leu	Pro	Arg	Leu 5	Ser	Phe	Asn	Asn	Val 10	Ser	Trp	Thr	Leu	Leu 15	Tyr
Tyr	Leu	Phe	Ile 20	Phe	Gln	Val	Arg	Gly 25	Glu	Asp	Ser	Gln	Lys 30	Ala	Val
Pro	Ser	Thr 35	Arg	Thr	Ser	Cys	Pro 40	Met	Gly	Ser	Lys	Ala 45	Tyr	Arg	Ser
Tyr	Cys 50	Tyr	Thr	Leu	Val	Thr 55	Thr	Leu	Lys	Ser	Trp 60	Phe	Gln	Ala	Asp
Leu 65	Ala	Cys	Gln	Lys	Arg 70	Pro	Ser	Gly	His	Leu 75	Val	Ser	Ile	Leu	Ser 80
Gly	Gly	Glu	Ala	Ser 85	Phe	Val	Ser	Ser	Leu 90	Val	Thr	Gly	Arg	Val 95	Asn

-continued

Asn	Asn	Gln	Asp	Ile	Trp	Ile	Trp	Leu	His	Asp	Pro	Thr	Met	Gly	Gln
			100					105					110		
Gln	Pro	Asn	Gly	Gly	Gly	Trp	Glu	Trp	Ser	Asn	Ser	Asp	Val	Leu	Asn
		115					120					125			
Tyr	Leu	Asn	Trp	Asp	Gly	Asp	Pro	Ser	Ser	Thr	Val	Asn	Arg	Gly	Asn
	130					135					140				
Cys	Gly	Ser	Leu	Thr	Ala	Thr	Ser	Glu	Phe	Leu	Lys	Trp	Gly	Asp	His
145					150					155					160
His	Cys	Asp	Val	Glu	Leu	Pro	Phe	Val	Cys	Lys	Phe	Lys	Gln		
				165					170						

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 165 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Rattus rattus*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Thr	Arg	Asn	Lys	Tyr	Phe	Ile	Leu	Leu	Ser	Cys	Leu	Met	Val	Leu
1				5					10					15	
Ser	Pro	Ser	Gln	Gly	Gln	Glu	Ala	Glu	Glu	Asp	Leu	Pro	Ser	Ala	Arg
			20					25					30		
Ile	Thr	Cys	Pro	Glu	Gly	Ser	Asn	Ala	Tyr	Ser	Ser	Tyr	Cys	Tyr	Tyr
		35					40					45			
Phe	Met	Glu	Asp	His	Leu	Ser	Trp	Ala	Glu	Ala	Asp	Leu	Phe	Cys	Gln
	50					55					60				
Asn	Met	Asn	Ser	Gly	Tyr	Leu	Val	Ser	Val	Leu	Ser	Gln	Ala	Glu	Gly
65					70					75				80	
Asn	Phe	Leu	Ala	Ser	Leu	Ile	Lys	Glu	Ser	Gly	Thr	Thr	Ala	Ala	Asn
				85				90						95	
Val	Trp	Ile	Gly	Leu	His	Asp	Pro	Lys	Asn	Asn	Arg	Arg	Trp	His	Trp
			100					105					110		
Ser	Ser	Gly	Ser	Leu	Phe	Leu	Tyr	Lys	Ser	Trp	Asp	Thr	Gly	Tyr	Pro
		115					120					125			
Asn	Asn	Ser	Asn	Arg	Gly	Tyr	Cys	Val	Ser	Val	Thr	Ser	Asn	Ser	Gly
	130					135					140				
Tyr	Lys	Lys	Trp	Arg	Asp	Asn	Ser	Cys	Asp	Ala	Gln	Leu	Ser	Phe	Val
145					150					155					160
Cys	Lys	Phe	Lys	Ala											
				165											

We claim:

1. A preparation of a naturally occurring mammalian islet neogenesis associated protein (INGAP protein) substantially free of other mammalian proteins.

2. The preparation of claim 1 wherein the INGAP protein has the amino acid sequence shown in SEQ ID NO: 2.

3. A preparation of a polypeptide which comprises a sequence of at least 15 consecutive amino acids of a naturally occurring mammalian islet neogenesis associated protein (INGAP protein), wherein said polypeptide has immunogenic activity.

4. The preparation of claim 3 wherein said polypeptide is a fusion of said sequence to a second polypeptide derived from a second protein.

55 5. The preparation of claim 3 wherein said polypeptide is conjugated to a second polypeptide.

6. The preparation of claim 3 wherein said polypeptide is conjugated to a solid support.

60 7. The preparation of claim 3 wherein said polypeptide has a biological activity of said mammalian INGAP protein.

8. The preparation of claim 7 wherein said biological activity is the ability to stimulate pancreatic duct cells to grow and proliferate.

65 9. The preparation of claim 3 wherein said polypeptide comprises amino acids #103 to #122 of the mammalian INGAP protein as shown in SEQ ID NO:2.

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* * * * *

DECLARATION BY THE INVENTORS

My residence, post office address and citizenship are as stated below next to my name, I believe I am an original, first and joint inventor of the subject matter that is described and claimed in letters patent number 5,834,590, granted on November 10, 1998, and for which invention I solicit a reissue patent on the invention entitled **INGAP PROTEIN INVOLVED IN PANCREATIC ISLET NEOGENESIS**, the specification of which as amended by the attached Preliminary Amendment is attached hereto.

(37 C.F.R. section 1.175)

I acknowledge the duty to disclose information that is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

In compliance with this duty, there is attached an information disclosure statement in accordance with 37 C.F.R. section 1.98.

PRIORITY CLAIM

I do not claim foreign priority benefits under Title 35, United States Code, section 119 of any foreign application(s) for patent. No such applications have been filed.

(37 C.F.R. section 1.175)

That I verily believe the original patent to be partly inoperative or invalid by reason of (37 C.F.R. section 1.175(a)(1)):

* the patentee claiming more or less than the patentee had a right to claim in the patent.

That the error listed above, which is being corrected, up to the time of the filing of this reissue declaration arose without any deceptive intention on the part of the applicant. (37 C.F.R. section 1.175(a)(2).

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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[illegible]

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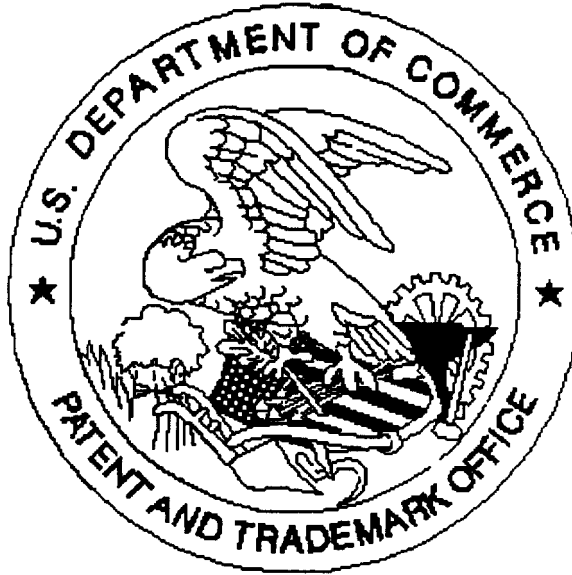
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ADDED PAGE

Figure 1 consists of 12 sub-graphs labeled (a) through (l), each showing the growth of *E. coli* O157:H7 in ground beef under different treatment conditions. The y-axis for all graphs is \log_{10} CFU/g, ranging from 0 to 10. The x-axis is time in hours, ranging from 0 to 120. The graphs show various growth curves, with some treatments showing significant inhibition of growth compared to the control.

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Application deficiencies found during scanning:

☒ Page(s) 5-6 of 6 of Transmittal were not present
for scanning. (Document title)

☒ Page(s) 4-6 of 6 of Declaration were not present
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